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Cholesterol implications on coconut liposomes encapsulation of beta-carotene and vitamin C

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Abstract. The implication of cholesterol on coconut liposomes encapsulation of beta-carotene and vitamin C was investigated through their encapsulation efficiency (*EE*) and partition coefficient (log P). In sole encapsulation the presence of cholesterol up to 40%s showed a decline in beta-carotene's *EE* while for vitamin C the *EE* was improved. The presence of cholesterol affects co-encapsulation both vitamin C and beta-carotene. The beta-carotene *EE* decreases as the cholesterol increases but vitamin C achieves optimum value at 90.05% in 20% cholesterol. The log P value of coconut phospholipids, vitamin C, cholesterol and beta-carotene were 4.87-11.2; -1.91; 7.11; and 11.12 respectively. The encapsulation efficiency and partition coefficient reveals that co-encapsulation of Vitamin C and beta-carotene in coconut liposomes is influenced by cholesterol because of their unique molecular lipophilicity.
Keywords: liposomes, cholesterol, vitamin C, beta-carotene, membrane, encapsulation, partition coefficient, Marvin Sketch, hydrophobicity, *Cocos nucifera L.*

1. Introduction

Beta-carotene and vitamin C (ascorbic acid) are important bioactive compounds for the human body. Both of them are needed because of their antioxidant properties [1, 2]. Antioxidant properties of beta-carotene and vitamin C play an important role in preventing various diseases such as heart disease, cancer, diabetes and cataracts [3, 4]. In spite of all that, beta-carotene and vitamin C have some drawbacks in their uses [5]. Beta-carotene has high hydrophobicity, low bioavailability and low chemical stability [6, 7]. The oxygen, high temperature, humidity and heavy metals causes vitamin C to be unstable and easily oxidized [1]. These deficiencies can be resolved by encapsulating beta-carotene and vitamin C in liposomes [2, 8]. The stability and bioavailability of beta-carotene can be amplified in liposomes [5]. Vitamin C can be protected from oxidation and increase its stability [1].

Liposomes are lipid vesicles that have the ability to carry either hydrophobic or hydrophilic active substances. Hydrophilic compounds will be inside the interior cavity of the liposomes while hydrophobic compounds will be in the liposome membranes. Liposomes are biodegradable, biocompatible, good safety profile, have low toxicity and small in size [9, 10]. The structure and nature of the liposomes cause vitamin C and beta-carotene suitable to be encapsulated in liposomes. Beta-carotene will be encapsulated in the liposome membrane. Vitamin C will be encapsulated in the interior cavity of the liposome [11].

The liposomes are build up from phospholipid molecules which are amphiphilic molecules [12, 13]. Phospholipids can be acquired from natural sources, including coconut meat and sesame seeds [10, 14]. In general, when manufacturing liposomes, the cholesterol is added. The addition of cholesterol will affect the rigidity of liposome membranes and so too their permeability [15].

Hudiyanti *et al.* [14] Hudiyanti et al. have used sesame liposomes to encapsulate beta-carotene and vitamin C. They found that cholesterol had different effects on the encapsulation of these two compounds. Cholesterol increases the vitamin C encapsulation but decreases beta-carotene. In different studies, Hudiyanti et al. have encapsulated vitamin C in coconut liposomes and the presence of cholesterol up to 30% increased its encapsulation efficiency [16]. In this paper we present the implications of cholesterol in coconut liposomes when encapsulating both vitamin C and beta-carotene either separately or concurrently. We discovered that concurrent encapsulation of both vitamin C and beta-carotene in coconut liposomes was influenced by cholesterol due to their different molecular hydrophobicity.

2. Materials and method

2.1. Materials

Coconut phospholipids isolated in house (with head groups composition were choline, ethanolamine and serine while the acyl tail were 22.75% dodecanoic acid, 14.60% hexadecanoic acid and 48.22% octadecanoic acid), cholesterol and beta-carotene from Sigma Aldrich, uncoated vitamin C from Brataco.

2.2. Encapsulation of Vitamin C and Beta-Carotene in Coconut Liposomes

Coconut liposomes were prepared based on method by Hudiyanti *et al.* [10]. Coconut liposomes were made with composition 0%, 10%, 20%, 30%, and 40% cholesterol. The manufacture of liposomes involved three stages, namely the manufacture of thin layers, hydration and sonication. Beta-carotene and vitamin C as active ingredients were encapsulated in coconut liposomes during the production. Beta-carotene, a nonpolar compound, with concentration C_i, was added to the phospholipids and cholesterol mixtures before thin layers formation. Vitamin C solution in a phosphate buffer, with concentration C_i, was added in the hydration process. The dispersion that formed was then centrifuged. The supernatant produced by centrifugation was analysed by UV-Vis spectrophotometer at a wavelength of 453 nm for beta-carotene and 265 nm for vitamin C. The encapsulation efficiency (*EE*) was measured to determine the ability of liposomes for encapsulating vitamin C and beta-carotene. *EE* values were calculated from the concentration of vitamin C and beta-carotene which was not encapsulated (C_f) as in equation 1.

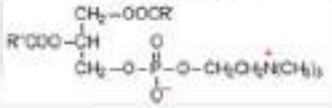
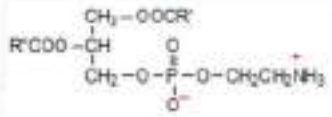
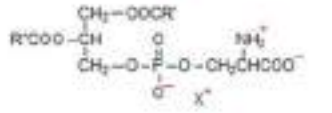
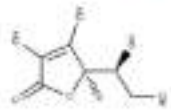
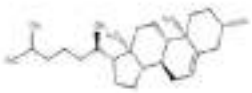
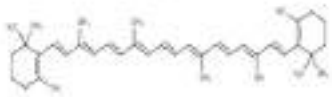
$$EE\% = \left(\frac{C_i - C_f}{C_i}\right) x\ 100\% \quad (1)$$

Co-encapsulation was performed with 20% of beta-carotene and 5% of vitamin C as the initial concentration [14, 16].

2.3. Determination of Partition Coefficient (Log P)

Log P of every component contained in coconut liposomes was calculated. Log P was calculated for coconut phospholipids, vitamin C, cholesterol and beta-carotene. Log P calculation was conducted based on its chemical structure [17] by Marvin Sketch application. For coconut phospholipids, log P was calculated based on the arrangement of head and tail composition as mention in the materials section. The chemical structures were presented in table 1 below.

Table 1. Chemical structures for partition coefficient calculation.

Compounds	chemical structures
Coconut Phospholipids: Phosphatidylcholine Acyl tail (R): dodecanoic acid, hexadecanoic acid, octadecanoic acid	
Coconut Phospholipids: Phosphatidylethanolamine Acyl tail (R): dodecanoic acid, hexadecanoic acid, octadecanoic acid	
Coconut Phospholipids: Phosphatidylserine Acyl tail (R): dodecanoic acid, hexadecanoic acid, octadecanoic acid	
Vitamin C	
Cholesterol	
Beta-carotene	

3. Results and discussion

3.1. Efficiency of Beta-carotene Encapsulation in Coconut Liposomes

Encapsulation efficiency (*EE*) shows the ability of coconut liposomes in encapsulating beta-carotene. The lower the *EE* value, the lower the ability of liposomes to encapsulate beta-carotene. Results of *EE* beta-carotene are presented in Figure 1. Figure 1 shows that the highest *EE* value of beta-carotene occurs at 0% cholesterol. The lowest *EE* value at 40% cholesterol. This shows that addition of cholesterol can reduce the *EE* of beta-carotene in coconut liposomes. The decline in *EE* occurs due to high competition between beta-carotene and cholesterol to be in the interior of the liposome membranes. From the molecular structure point of view, see table 1, cholesterol is more adaptable within the liposome membranes than beta-carotene since the cholesterol structure is smaller and more flexible than beta-carotene [18]. Cholesterol has an amphiphilic structure such as phospholipid which makes it easier to slip in the membrane and orient itself in line to the phospholipid molecule whereas beta-carotene is a a bulky stiff nonpolar molecule that will make it more difficult to go to the nonpolar part of the membrane. As a result cholesterol molecules will block beta-carotene from reaching the nonpolar part of the membrane. Therefore, an increase in cholesterol concentration has induced a reduction of the beta-carotene encapsulation and the *EE* beta-carotene decreases.

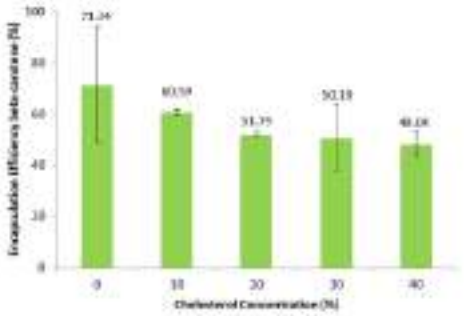


Figure 1. Encapsulation efficiency of beta-carotene in coconut liposomes.

3.2. Efficiency of Vitamin C Encapsulation in Coconut Liposomes

The encapsulation efficiency value (*EE*) shows the ability of liposomes in encapsulating vitamin C. The higher the *EE*, the better the ability of coconut liposomes to encapsulate vitamin C. The *EE* of vitamin C in coconut liposomes is presented in Figure 2. The graph shows that the *EE* increases as the cholesterol concentration increase. This is slightly different from the previous research by Hudiyanti *et al.* [16] which states that the encapsulation efficiency of vitamin C rises up to maximum at 30% cholesterol and then decreases at 40% cholesterol. We assume it dues to the differences of the phospholipid acyl chains. Our phospholipids are dominated by octadecanoic acid while the previous research are dodecanoic acid. The differences in the composition of the acyl chain is common for phospholipids derived from nature since they are a mixture of various phospholipid species that have similar polarity.

EE of vitamin C in coconut liposomes increases after the addition of cholesterol. This increase in *EE* value is affected by the rigidity (stiffness) of the bilayer membrane. Cholesterol makes the bilayer membrane more rigid [10, 13]. Adding cholesterol to the liposome bilayer membranes causes the phospholipid tail groups become more regular. As a result cholesterol occupies the liposome membrane pores easily so its permeability decreases [10]. Liposomes entrap vitamin C better when there is cholesterol inside the membrane.

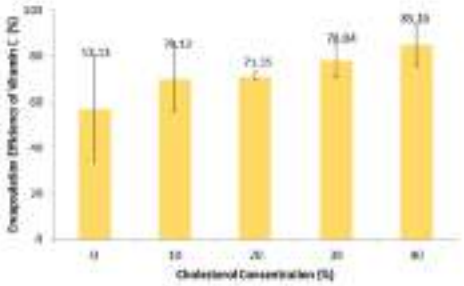


Figure 2. Encapsulation efficiency of vitamin C in coconut liposomes.

3.3. Efficiency of Vitamin C and Beta-carotene Co-encapsulation in Coconut Liposomes

The *EE* in co-encapsulation of vitamin C and beta-carotene in coconut liposomes are presented on Figure 3. The EE of beta-carotene reduces as the cholesterol concentration increases. The lowest *EE* occurs at 40% cholesterol. The highest *EE* occurs when cholesterol is not present inside the liposome membranes. Similar results are displayed in the absence of vitamin C, figure 1. Beta-carotene is a nonpolar compound while vitamin C is a polar compound. These opposite properties suggest that they will reside in different region of liposomes after encapsulation process. Beta-carotene is in the liposome membranes while

vitamin C is in the liposome core. This way beta-carotene and vitamin C will not interfere to each other during encapsulation. The results confirm that cholesterol has negative effect on beta-carotene encapsulation whether there is vitamin C in the vicinity or not.

Compare to single encapsulation of vitamin C (see Figure 2) different results are uncovered from *EE* of vitamin C in co-encapsulation with beta-carotene in Figure 3. Addition of cholesterol increase *EE* of vitamin C up to 90.05% at 20% cholesterol then goes down for the remaining concentration. Results signify that both cholesterol and beta-carotene in the liposome membranes affect the encapsulation of vitamin C. Cholesterol increase vitamin C encapsulation by sealing the membrane pores but beta-carotene presence causes cholesterol blockage disrupted. Bulky beta–carotene molecules will be in the centre of the membranes. The two rings of beta-carotene will interact with the cholesterol rings tail. The beta-carotene and cholesterol will push each other causing the membrane to stretch. The liposome membranes become more delicate and cholesterol cannot seal the pores properly. The more the cholesterol in the membranes the wider the pores opened. We believe that 20% cholesterol is the optimum concentration of cholesterol to be in balance with 20% beta-carotene inside the membranes.

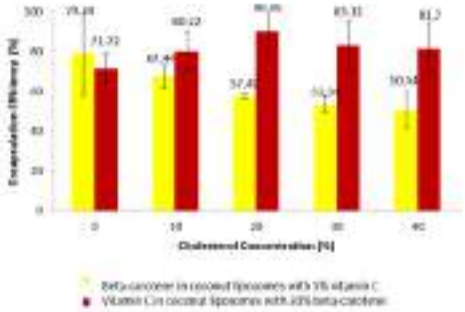


Figure 3. Encapsulation Efficiency of Co-encapsulation of Vitamin C and Beta-carotene in Coconut Liposomes.

3.4. Determination of Partition Coefficient (Log P)

The partition coefficient (log P) is done to determine the inclination of a compound to be in the lipophilic or hydrophilic phases [19]. Compounds with negative log P values are hydrophilic and will be dispersed in the water phase. Compounds with log P positive are lipophilic and will be dissolved in the organic phase [19, 20]. For liposomes it means that lipophilic compounds are in the liposome membranes and hydrophilic compounds are in the interior cavity of the liposomes. The log P value of coconut phospholipids, vitamin C, cholesterol and beta-carotene is presented in Table 2. The greater the log P value, the greater the lipophilicity and the lower the hidrophilicity [20-22].

Log P of Coconut phospholipids are in the range of 4.87-11.2. These values are the partition coefficient of the phospholipid molecules. However, phospholipid itself is an amphiphilic molecule with a hydrophilic head group. This makes phospholipids as amphiphilic compounds which tend to be lipophilic. Phospholipids in the aquos medium (hydrophilic) will easily undergo self-assembly to form liposomes with their head groups facing toward the hydrophilic medium. The beta-carotene has log P 11.12. It is in the region of the coconut phospholipid log P which makes coconut phospholipids suitable to be used as raw materials for making liposomes to encapsulate beta-carotene. Whereas the log P value of vitamin C is -1.91 indicating that vitamin C is hydrophilic. Vitamin C will be in the interior cavity of the liposomes.

Table 2. The Partition coefficient of liposomes components

Compounds	Partition Coefficient (log P)	Properties
Coconut Phospholipids: Phosphatidylcholine Phosphatidylethanolamine Phosphatidylserine Acyl tail (R): dodecanoic acid hexadecanoic acid octadecanoic acid	4.87 – 11.2	Lipophilic
Vitamin C	-1.91	Hydrophilic
Cholesterol	7.11	Lipophilic
Beta-carotene	11.12	Lipophilic

In table 2 we see cholesterol and beta-carotene are both lipophilic compounds base on the log P values. They therefore are in the lipophilic part of liposome membranes. They will be in each other spot and overcrowded to occupy the membranes. The greater log P value of beta-carotene makes beta-carotene more preferable in membrane liposomes than cholesterol. The log P confirms our experimental results obtained above. It is also in agreement with the research conducted by Socaciu et al. that the positions of cholesterol and beta-carotene are in the liposome membranes [23].

4. Conclusion

In single encapsulation, beta-carotene encapsulation efficiency decreases with the addition of cholesterol while vitamin C increases. The presence of cholesterol influences co-encapsulation of vitamin C and beta-carotene. The beta-carotene encapsulation efficiency decreases with cholesterol up to 40%. However, vitamin C encapsulation efficiency reach the optimum value of 90.05% when cholesterol concentration attain 20%. Encapsulation efficiency and partition coefficient of the compounds of interest indicate that co-encapsulation of vitamin C and beta-carotene in coconut liposomes is affected by cholesterol due to the different molecular lipophilicity.

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